World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.org/
Original Article

Phytochemical screening and standardization of polyphyto mixture for diabetes

Anuj Nautiyal[*1], N.V. Satheesh Madhav[1], S. Bhattacharya [2], S.N. Uniyal[3]

[1]Faculty of Pharmacy, DIT University, Dehradun, [2]Global Institute of Pharmaceutical Education and Research, Kashipur, Uttarakhand, [3]Surya Hospital, Dehradun, Uttarakhand, India

Received: 20-10-2014 / Revised: 12-11-2014 / Accepted: 18-11-2014

ABSTRACT

Standardization of the Polyphyto formulations is important for determining the quality of drugs, based on the concentration of their active constituents, physical and chemical standards. This article reports on standardization of a Polyphyto mixture used as anti-diabetic. Specific morphological parts of the plants were used in the Polyphyto mixture. Polyphyto mixture has been standardized on the basis of Organoleptic properties, physical characteristics, and physicochemical properties.

Key words: Standardization, Polyphyto, Anti-diabetic, Organoleptic, Physicochemical, fluorescence

INTRODUCTION

Diabetes mellitus (DM) is a disorder of metabolism characterized by hyperglycemia, glycosuria, and negative nitrogen balance with sometime can cause ketonemia. The major types of diabetes classified are: Type 1 and Type 2. Type I is insulin dependent diabetes mellitus also called as juvenile onset diabetes mellitus. Type II is noninsulin-dependent diabetes mellitus also called as maturity onset diabetes mellitus [1]. The assessment of quality of herbal formulations is most important for justification of their acceptability in this modern system of medicine.

One of the frequently occurring problems in the herbal industry is the unavailability of rigid quality control parameters for herbal materials and their formulations. So, the regulatory bodies developed the standardization procedures and various specifications for Ayurvedic mixtures**.** Department of AYUSH, Government of India, have launched a scheme to develop a standard operating procedure for the manufacturing process to develop the pharmacopeia standards for the Ayurvedic formulations [2]. There is lot of things to know and many contradictory theories relating to the subject of herbal formulations and their relationship with our body system. India has a potential to emerge as the major country and can lead as major role in production of standardized, effective herbal formulations. There are essential needs to explore the medicinally important plants in India. This can be only be achieved if the herbal formulations products are properly evaluated and analyzed by using sophisticated modern techniques of standardization. The World Health Organization (WHO) has appreciated the value of medicinal plants for improving the health of people in developing nations and has made certain guidelines for supporting the national policies on herbal medicine and for study of its potential use including their evaluation, safety, and efficacy [3]. It is important to make an effort towards standardization of the herb material to be used as therapy. The process of standardization of herbal can be achieved by stepwise pharmacognostic studies [4].

MATERIAL AND METHODS

Physico-chemical studies like total ash content, water soluble ash value, acid insoluble ash value, water and alcohol soluble extract value, loss on drying and successive extractive value by Soxhlet extraction method were carried out as per the WHO guide lines [5]. The preliminary photochemical screening was performed as per the standard methods [6].

Plant material: Polyphyto mixture consists of 8 ingredients, viz., *Cinnamomum zeylanicumm, Astragalus gumifer, Murraya koenigii, Ocimum sanctum, Musa paradisica, Paspalum scrobiculatam, Plantago ovata, Avena sativa*. All these plants used in the Polyphyto mixture were

***Corresponding Author Address: Anuj Nautiyal,** Faculty of Pharmacy, DIT University, Dehradun, Uttarakhand, India; E-mail: anujnautiyal@rediffmail.com*

collected from the local field of Dehradun district of Uttarakhand. These plants were authenticated by Prof (Dr.) R.C.Dubey, Department of Botany and Microbiology, Gurukul Kangri, Vishwavidyalaya, Haridwar.

Preparation of Polyphyto mixture: All the ingredients used in Polyphyto mixture (Table-1) were collected and subjected to shade drying for about 8 weeks. The dried plant material was further crushed to powder and passed through 100 # sieve to obtain fine powder. Each of the powders was taken in different proportions as per quantity required for the mixture and thoroughly mixed together by geometrical mixing to get a homogenous mixture, stored in air tight container for further use.

Standardization parameters: The various standardization parameters studied were Organoleptic properties, Physico-chemical investigations, Fluorescence analysis, Preliminary Phytochemical analysis, determination of moisture content, swelling factor, surface tension and density, determination of crude fat and determination of physical characteristics of powder mixture.

Organoleptic evaluation: The Organoleptic characters [7] of the samples were determined based on the method described by Siddiqui *et al*. Organoleptic evaluation refers to evaluation of the mixture by color, odor, taste and texture etc.

Physicochemical investigations: Physico-chemical investigations of formulations were carried out were the determination of extractive values and ash values [8].

Determination of pH: 1% solution of Polyphyto mixture was prepared in distilled water and pH was determined by using the pH meter SYSTRONICS DIGITAL pH METER, MK VI.

Fluorescence analysis: Fluorescence characters of Polyphyto mixture was determined by using different chemical reagents under ordinary and ultraviolet light [9]. For this about 1 mg of the sample was taken in a glass slide and treated with different reagents for the presence of their fluorescence characters under ultra-violet lamp.

Preliminary Phytochemical analysis: Preliminary qualitative Phytochemical analysis of all the extracts was carried out by employing standard conventional protocols [10-12].

Determination of moisture content: Moisture content was determined by loss on drying method [13]. And for this about 3 gm of the weighed quantity of the Polyphyto mixture was taken and kept in oven at 105^0C till a constant weight was obtained. The amount of moisture present in the sample was calculated as reference to the air dried drug.

Determination of swelling factor: The swelling factor is estimated for the amount of mucilage present in the drug. The technique has been accepted as an official method for evaluation by various pharmacopoeias. One gram of the Polyphyto mixture was taken and kept for 24 hours in a graduated, stoppered cylinder, in contact with the water up to the mark of 20ml. After 24 hours the increase in volume was noted [14].

Determination of viscosity, surface tension and density: Density, surface tension and viscosity of the 1% aqueous Polyphyto mixture was estimated [15] (Table 6).

Determination of crude fat: 2 g of moisture free Polyphyto mixture with petroleum ether in Soxhlet extractor, for 6 h till a drop taken from the drippings left no greasy stain on the filter paper. After boiling with petroleum ether[16], the residual petroleum ether was filtered and filtrate was evaporated in a pre weighed beaker. Increase in weight of beaker gave the crude fat [17].

Determination of physical characteristics of Polyphyto mixture: Physical characteristics like bulk density, tap density, Haussner ratio, and Carr's consolidation index were determined for the Polyphyto mixture [18, 19] .The term bulk density refers to packing of particles or granules. The volume of packing can be determined in an apparatus consisting of graduated cylinder mounted on mechanical tapping device (jolting volumeter) that has a specially cut rotating can. 100 grams of weighed Polyphyto mixture was taken and carefully added to cylinder with the help of funnel. The initial volume was noted and sample was then tapped until no further reduction in volume was noted. The initial volume gave the bulk density value and after tapping the volume reduced, it gives the value of tapped density. Haussner ratio is related to interparticle friction and as such can be used to predict the powder flow properties. Carr's index is a method of measuring the powder flow from bulk density [20].

RESULTS AND DISCUSSION

Polyphyto mixture was subjected to various analytical techniques. Botanical parameters revealed that the mixture was buff-white in color, with characteristic odor, tasteless, and fine texture (Table-2).

Table-1: Composition of Polyphyto mixture (FD18)

S.No.	Name of plant	Part used	Family	Strength (%)
1	*Cinnamomum zeylanicumm*	Bark	Lauraceae	2
2	*Astragalus gumifer*	Gum	Leguminoseae	2
3	*Murraya koenigii*	Leaf	Rutaceae	2
4	*Ocimum sanctum*	Leaf	Lamiaceae	4
5	*Musa paradisica*	Stem	Musaceae	10
6	*Paspalum scrobiculatam*	seed	Poaceae	10
7	*Plantago ovata*	Husk	Plantaginaceae	20
8	*Avena sativa*	seed	Poaceae	50

Table2: Organoleptic properties of Polyphyto mixture

Appearance	Color	Odor	Taste	Texture	Particle size
Powder	Buff-white	characteristic	Tasteless	Fine	Fine 100#

Results of quantitative analysis for Total ash (2.46±0.05), Acid insoluble ash (0.5 ± 0.07) , Water soluble ash (6.19 ± 0.41), Alcohol soluble extractives (18 ± 0.02), Water soluble extractive (15 ± 0.03),Hexane soluble extractive (5 ± 0.01), Chloroform soluble extractive (6.4 ± 0.05), Petroleum ether soluble extractive (3.6± 0.46), Particle size (100 mesh), Loss on drying at 105º C was found to be (6.9 ± 0.3) , pH (1% aq. Soln.),Crude fat were calculated and results were shown (Table-3). Ash value is useful in determining authenticity and purity of drug and also these values are important quantitative standards. Percent weight loss on drying or moisture content was found to be 6.9% w/w. The less value of moisture content could prevent bacterial, fungal or yeast growth. The results of fluorescent studies of the powdered plant material using different chemical reagents were studied and a given in (Table-4). Fluorescence is an important phenomenon exhibited by various chemical constituents present in plant material. If the substances themselves are not fluorescent, they may sometimes be converted into fluorescent substances by reagents so some crude drugs are often assessed qualitatively in this way and it is an important parameter of Pharmacognostical evaluation.

Table-3: Physiochemical characteristics of Polyphyto mixture

S.No.	Parameters	**Percentage mean (n=3) ± SD**
1	Water soluble extractive (w/w %)	15 ± 0.03
2	Alcohol soluble extractive (w/w %)	18 ± 0.02
3	Hexane soluble extractive (w/w %)	5 ± 0.01
4	Chloroform soluble extractive (w/w %)	6.4 ± 0.05
5	Petroleum ether soluble extractive (w/w %)	3.6± 0.46
6	Ash content (w/w %)	2.46±0.05
7	Acid insoluble ash (w/w %)	0.5 ± 0.07
8	Water Soluble ash (w/w %)	6.19 ± 0.41
9	Particle size	100 # mess size
10	Moisture content	6.9 ± 0.3
11	pH	7 .32 ± 0.1
12	Crude fat	0.2 ±0.1

Table-4: Fluorescence analysis

	FD18	
Chemicals used	**Ordinary light**	**U.V. light**
Powder +Acetic acid	grey	White
Powder +Conc. HNO_3	Reddish-brown	Light-green
Powder +Iodine sol.	Black	Greenish-black
Powder +Br. water	Whitish-grey	Grey
Powder +5% H_2O_2	Whitish-grey	Grey
Powder +NH_3 Sol.	brown	Green
Powder +Dil.H_2SO_4	Whitish-grey	Grey
Powder +Dil. HNO_3	Brown	Green
Powder +5%NaOH	Yellow-brown	green
Powder +Conc. HCL	Greenish-brown	Light-green
Powder +$FeCl_3$	Greenish-brown	Green
Powder +Pot.chromate	Yellow	Light green
Powder +Dil. HCL	whitish-brown	Grey
Powder +Conc.H_2SO_4	Reddish-Brown	Dark green
Powder +Pot. dichromate	Orange-brown	Fluorescence green

The results of preliminary phytochemical analysis of are given in (Table-5). Density, viscosity and surface tension of the Polyphyto mixture (1% aq.) were determined and results were tabulated (Table-6). Physical properties of the Polyphyto mixture, like bulk density, Tap density, Carr's compressibility index, Haussner's ratio, were determined and results were tabulated (Table-7). Swelling factor of the Polyphyto mixture was determined it show that mucilage is present; indicating the presence of mucilage in the formulation which is also revealed in the Phytochemical screening indicating the presence of the mucilage in the Polyphyto mixture.

Table-5: Phytochemical screening

S.No.	Phytoconstituents	Name of test	Results
1	Carbohydrates	Molischs test Barfoeds test Benedicts test Fehlings test	+++
2	Alkaloids	Mayers test Hagers test Wagners test	+
3	Tannins	Ferric chloride test Lead acetate test Match stick test	+
4	Proteins and amino acids	Heat test Ninhydrin test Millions test Biuret test	++
5	Flavanoids	Zinc chloride test Shinoda test	++
6	Glycosides	General test	+
7	Saponin glycosides	Froth forrmation test	+
8	Mucilage and gums	Alcoholic ppt. test Ruthenium red test	++
9	Volatile oil	Hydrodistillation PET sprit test	+
10	Starch	Iodine test	+
11	Lignin	HCl +Phloroglucinol	---
12	Inulin	α-napthol and sulphuric acid	---

+ + + = Intense; + + = Moderate; + = Slight; - - - : Absent

Table-6: Density, viscosity and surface tension

Parameter	Values
Density (1% soln.)	1.01
Viscosity (1% soln.) 1.01 cp	1.35cp
Surface tension (1% soln.)	43.52

Table-7: Physical properties of Polyphyto mixture

Parameter	Values
Bulk density	0.398 gm/mL
Tap density	0.497 gm/mL
Carr's compressibility index	19.91
Haussner's ratio	1.247

CONCLUSION

Polyphyto mixture for treatment of diabetes was formulated by using various anti-diabetic plant parts in a fixed proportions .The prepared mixture was then screened by various standardization parameters as per Ayurvedic pharmacopoeia standards. The research out comings of the standardization parameters can be further used for evaluating the quality and purity of the other Polyphyto mixture.

Acknowledgment

We are thankful to the Chairman of The DIT University, Faculty of pharmacy, Dehradun; Uttarakhand, India supported me to conduct work and providing a laboratory facility and infrastructure.

REFERENCES

1. Tripathi KD. *Essentials of Medical Pharmacology,* 6th ed.; Jaypee Brothers: New Delhi, 2009.
2. Kalaiselvan V et al.Quality assessment of different marketed brands of Dasamoolaristam, an Ayurvedic formulation. Int. J of Ayurveda Res 2010; 1(1): 10-13
3. Organisation Mondiale De La Sante, Quality control methods for medicinal plant materials, 559, rev.1, Original English, World Health Organisation, 1992: 159
4. Ozarkar K.R. Studies on anti-inflammatory effects of two herbs Cissus quadrangularis Linn. And Valeriana wallichi DC using mouse model. Ph.D. Thesis, University of Mumbai:Mumbai, 2005
5. Quality Control Methods for Medicinal Plant Materials, World Health Organisation, Geneva, 1998, 25-28.
6. Harborne JB. Phytochemical Methods. Jackman H. (Ed.),London, 1973, 70.
7. Siddiqui Hakim MA. Format for the pharmacopoeial analytical standards of compound formulation, workshop on standardization of Unani drugs, (appendix), 24-25 January. New Delhi: Central Council for Research in Unani Medicine (CCRUM); 1995.
8. Indian Pharmacopoeia, Ministry of Health and Family Welfare. New Delhi: Government of India; 1996.
9. Chase CR, Pratt RJ. Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. J. Amr. Pharm. Assoc 1949; 38: 324- 331.
10. Trease E.G., Evans W.C. *Pharmacognosy*, 11th ed.; Balliere Tindall: London, 1978; pp. 115-222.
11. Sazada S et al. Preliminary Phytochemical analysis of Some Medicinal and Aromatic Plants.Adv. In Biological Res 2009; 3(5-6): 188-5.
12. Kokate C.K., Purohit A.P. and Gokhale S.B.Pharmacognosy, 34th ed.; Nirali Prakashan: Pune, 2006.
13. Mukherjee P.K. Quality control of herbal drugs, 1st ed.; Business horizons publishers: 2002: pp.195-196.
14. Kokate CK. Practical Pharmacognosy, 4th ed.; Nirali Prakashan: Pune,1994: pp.127.
15. Sinko Patrick J. *Martin`s Physical Pharmacy and Pharmaceutical Sciences*, 5th ed.; Lippincott Williams and Willkins: Philadelphia, 2006.
16. Chopra SL, Kanwar J S .In Analytical Agricultural Chemistry, 4th ed.; Kalyani Publications: New Delhi, 1991:pp. 297
17. Indrayan AK et al. Determination of nutritive value and analysis of mineral elements for some medicinally valued plants from Uttaranchal. Curr. Sci, 2005; 89, pp.1252-1255.
18. Lachman L, Lieberman H A, Kanig JL. The theory and practice of industrial pharmacy. 3 rd ed.; Varghese Publishing House; Mumbai, 1987: pp. 183-316.
19. Aulton ME. Pharmaceutics, The science of dosage forms designs. 2nd ed.; Churchill Livingston: New Delhi, 2002: pp.205-21.
20. Sriwastava NK et al .Standardization of Ajmodadi churna, a polyherbal formulation. J Pharmacog Res 2010; 2(2): 98-101.
21. African Pharmacopoeia.*General methods for analysis*, 1st edt.; Vol. 2: (OAU/STRC) Lagos,1986: pp.123.
22. Kamil MS, Paramjyothi S. Preliminary Pharmacognostical and Phytochemical Evaluation of Portulaca quadrifida Linn. Int. J PharmTech Res 2010; 2(3): 1699-1702
23. Ansari S.H. *Essentials of Pharmacognosy*. 1st ed.; Birla Publications: New Delhi, 2006.